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RESEARCH ARTICLE

Determination of Mango Variety Volatile Compounds and the Behaviour of *Sternochetus frigidus* (Mango Pulp Weevils)

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ABSTRACT

Sternochetus frigidus, commonly known as the mango pulp weevil, infests and damages the mango fruit, reducing both the quality and quantity of the harvest. The current study evaluated the infestation of this weevil and its influence by the volatile compound of the mango species: *Mangifera indica* (mangga epal) and *M. odorata* (kuinin). Mangoes were sliced open longitudinally to detect the presence of mango weevils. The mango peel and pulp were extracted for volatile compound determination using gas chromotography-mass spectrometry (GC-MS) headspace concentration methods. Results demonstrated a higher number of larvae and adults of *S. frigidus* on *M. odorata* (100%) than *M. indica* (70%). The higher infestation level was demonstrated by *M. odorata*, which had higher amine and monoterpene hydrocarbon concentrations. The study found only *M. indica* contained phenolandrene and copaene compounds, which were not found in *M. odorata*. *Mangifera odorata* contained linalool, limonene, and terpinene which was not found in *M. indica*. These compounds can be good pheromone-trapping lures for controlling mango pulp weevils.

1. INTRODUCTION

Mango, *Mangifera indica* (family Anacardiaceae), is the most popular fruit in the subtropical and tropical Asian regions. Mangoes taste deliciously and can be used in various foods and drinks. In Malaysia, the mango harvest season spans from March to May and August to October. There are different cultivars of mangoes each with its flavour such as Epal, Siku Raja, Chok Anan and Harumanis that are planted in Malaysia. Another mango species from the same family is *M. odorata* locally known as kuinin or kwini. *Mangifera odorata* is a cross hybrid between *M. indica*, commonly known as mango, and *M. foetida* locally known as bachang or macang. The *M. odorata* has appealing orange flesh and a distinct, potent scent. This fruit emits a strong smell that draws a variety of insects. This mango species is seasonal and seldom found in the market. However, *M. odorata* is much loved by the locals as a lunch meal with other dishes and served on a fruit platter. Mangoes contain various good nutrients for humans such as vitamins A, B6, vitamin C, K, magnesium, potassium and fiber.

The fruits produced have a substantial economic impact on Malaysia. Numerous issues affect mango production in plantations, including varying yields from year to year, uneven fruit quality at harvest, and vulnerability to pests and diseases. This crop's pest and disease community structure is significantly impacted by factors like commercial mango production, shifting crop management practices, expanding into new regions, increasing chemical treatments, and variety substitution. In addition, other new pests may emerge because of climate change. Pests and diseases also may spread more quickly among different mango-growing locations, especially if sufficient sanitary measures are not taken. Mangoes are easily attacked by pests such as scales, mealybugs, thrips (*Haplothrip pictipes*), gold dust weevil (*Hypomeces squamosus*) and mango *leaf-cutting weevil (Deporaus marginatus*) that feed on mango leaves. Those insects will eat the mango tree leaves and young shoots, causing the leaves to turn yellow, dry and fall.

Recently, the mango pulp weevil, *Sternochetus frigidus* (Coleoptera: Curculionidae) has become one of the most serious pests in mango plantations in Malaysia besides fruit flies (*Bactrocera* spp.). *Sternochetus frigidus* is a serious exotic insect pest that burrows into the flesh of mangoes thus the fruits are damaged resulting in the mango pulp being mushy and unattractive to eat, making the mangoes unmarketable. This situation was a major challenge for the industry. The mango pulp weevil infestation can only be detected within the mango fruit, not on the outside. As a result, pulp weevil infestation is undetectable. This weevil infests only the mango pulp, leaving no visible signs on the fruit's peel. As soon as the pulp weevil emerges from the tunnel, made through the mango flesh. An early attack by the weevil has caused premature fruit drop.

The mango pulp weevil can be controlled by using mechanical and chemical approaches by putting the mangoes in a bag or covering it completely while still attached to the branch so that the weevils are unable to infest the fruits. This weevil also can be controlled by spraying insecticides such as deltamethrin, fenitrothion, dimethoate and cypermethrin. However, these mango pulp weevil attacks still happen and have not been fully controlled. Thus, more information needs to be looked for to increase the understanding of this weevil population. It is believed that the volatile compound of the mango is the attractant to the weevil. *Mangifera indica*, or the mango tree, produces various volatile compounds that can play a role in pest behavior, including the attraction or deterrence of insects like the mango pulp weevil (De Jesus et al, 2004). There are a few volatile compounds in mango fruits that will attract the mango pulp weevil to lay eggs on the mangoes (Louella et al, 2020; Musharraf et al, 2016; Zhao et al, 2016). In particular, ripe mangoes release esters, terpenes, and alcohols that give them their characteristic aroma. Many mangoes variety have volatile components such as decane, 2-methyl heptanone, ethyl benzoate and linalool (De Jesus et al., 2004). Knowledge and understanding of the mangoes' volatile compound can help to develop effective control of this weevil by developing plant volatile-based attractant. Therefore, this study was conducted to study the interaction of mango pulp weevil, the *S. frigidus* in two types of mangoes, *M. indica* and *M. odorata,* with the volatile compounds of *M. indica* and *M. odorata* that are responsible for the infestation of *S. frigidus*.

2. MATERIALS AND METHODS

Two species of mango; *M. indica* (mangga epal) and *M. odorata* (kuinin) were chosen for this study as these mangoes are easily found and often planted in the home area. Fifty mango fruits of *M. indica* and another 50 mango fruits of *Mangifera odorata* (Kuini) were selected randomly around Penang Island. Mango fruits were selected randomly from mango trees at Teluk Kumbar, Farlim and in School of Biological Sciences, USM, Penang from March to May 2023. All fruit was brought to the lab for further processing and the identification of the mango pulp weevil. Fruits were sliced open longitudinally to detect the presence of mango weevils. Weevils were identified to species level following key references by Oberprieler (2008) and Poonchaisri & Chaowalit (2008).

For volatile compound determination, the mango peel was peeled off using a knife and the mango pulp was cut into smaller size before being ground into a fine texture. Each mango pulp (8 gm) was transferred into a 12 ml vial were sealed immediately by using 20 mm crimp top caps with the manual crimper. For the volatile compound determined from the fruit peels, each mango species, the skin was cut into 1 mm in width and 1 cm in length weighing 8g of mango peel. Then, each fruit peels were transferred into a 12 ml vial, sealed using 20 mm crimp top caps with the manual crimper. The volatile compounds in the pulp and peel of *M. indica* and *M. odorata* were extracted by using gas chromotography-mass spectrometry (GC-MS) Headspace concentration methods. Analysis was conducted using a GCMS-QP2010 Ultra Gas Chromotography-mass Spectrometry equipped with a Teledyne Tekmar HT3™ Headspace Autosampler in Main Equipment Laboratory, School of Biological Sciences. USM. . The mango samples (mango pulp and peel) vials were pressurized for 2 minutes and injected for 1 minute. The aroma components of headspace were carried along with the helium flow and then trapped together at 10 psi pressure and 100°C transfer line temperature. Mango's volatile aroma headspace was recorded for 20 minutes. Volatile compounds were identified by comparing flavour detected in mango pulp and peel samples over retention time (minutes) with standards in the library of flavour and fragrance. Results were presented in area normalization of the flavour detected and illustrated using a chromatogram graph.

3. RESULT AND DISCUSSION

Three stages of *S. firgidus* were found in the pulp of the mangoes (Table 1). Larva and adult stages were found during the dissection of mango fruits. In *M. indica*, there were 41 larvae, and 87 adults while in *M. odorata*, there were 253 larvae, and 239 adults. The infestation of mango pulp weevil was found greatest in *M. odorata* which showed 100% infestation compared to *M. indica* (70%).

Table 1: Mean number of mango pulp weevil (*S. firgidus*) of *M. indica* (n=50) and *M. odorata* (n=50).

Mango	Mean ± SE		Fruit infestation (%)
	larva	Adult	
***M. indica* (epal)**	2.5±0.21	6.4±0.15	70
***M. odorata* (kuinin)**	21.2±0.35	17.3±0.20	100

Identified constituents with the percentage of contributions of *M. indica* and *M. odorata* are listed in Tables

2 and 3 while Figure 1 shows the results of the Gass chromatogram. Ten volatile compounds were identified and profiled in *M. indica* pulp while 11 were positively identified in *M. odorata* pulp. Argon and ethanol were dominant volatiles in both pulps of *M. indica* and *M. odorata*. For *M. indica*, Argon comprised 73% and ethanol (22.1%). In *M. odorata*, the content of Argon was 52.4% while ethanol was 32.7%. According to DOAUS (1963), insect attractants were identified as alpha-pinene, myrcene, cymene, limonene and terpinolene. *Mangifera indica* pulp only recorded alpha-pinene (0.2%) whereas *M. odorata* has more insect attractants in its pulp such as alpha-pinene (3.5%), myrcene (2.9%), and limonene (0.1%).

Table 2: Volatile compounds in the pulp of *M. indica*.

No.	Compound	Retention time (minute)	Concentration (%)
1	N-dl-Alanylglycine	0.224	2.4
2	Argon	1.696	73
3	Ethanol	1.868	22.1
4	Alpha.-Pinene	7.984	0.2
5	2',6'-Dihydroxyacetophenone, bis(trimethylsilyl) ether	10.324	0.2
6	1,3,6-Octatriene, 3,7-dimethyl-,(Z)	12.472	0.5
7	Cyclopentasiloxane, decamethyl-	17.41	0.6
8	Cyclohexasiloxane, dodecamethyl-	25.488	0.2
9	Diethyltoluamide	36.249	0.8
10	2-Propenoic acid, 2-methyl-,dodecyl ester	43.525	0.1

Table 3: Volatile compounds in the pulp of *M. odorata* .

No.	Compound	Retention time (minute)	Concentration (%)
1	dl-Alanyl-l-alanine	0.1	1.8
2	Argon	1.7	52.4
3	Ethanol	1.868	32.7
4	Ethyl Acetate	2.302	0.3
5	Alpha-Pinene	7.989	3.5
6	beta.-Pinene	9.572	0.5
7	Myrcene	10.081	2.9
8	Cyclotetrasiloxane, octamethyl-	10.332	0.4
9	Limonene	11.654	0.1
10	1,3,6-Octatriene, 3,7-dimethyl-, (E)	12.029	0.2
11	Linalool	15.226	0.5

More volatile compounds were recorded from the peel of *M. indica* and *M. odorata* compared to its peel (Figure 2). The peel of *M. indica* exhibited 12 different volatile compounds (Table 4) while *M. odorata* had 13 volatile compounds from its peel (Table 5). The ethylamine was recorded greatest in the peel of both mangoes with *M. indica* (56.1%) and ethanol (16.3%), respectively. Meanwhile, *M. odorata* recorded 46.5% ethylamine and 12.5% ethanol. It was found that the peel of *M. odorata* had four insect attractants: alpha-pinene, beta-pinene, myrcene and limonene. Besides, ethyl acetate and ethyl ester are also found in *M. odorata* peel.

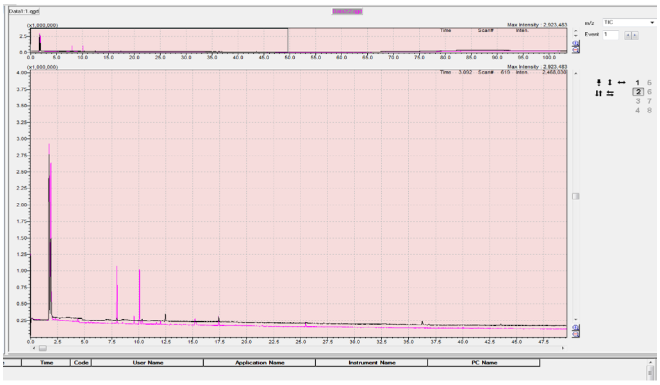

Figure 1: Gas chromatogram of volatile compounds in *M. indica*.(black line) and *M. odorata* (pink line) pulp.

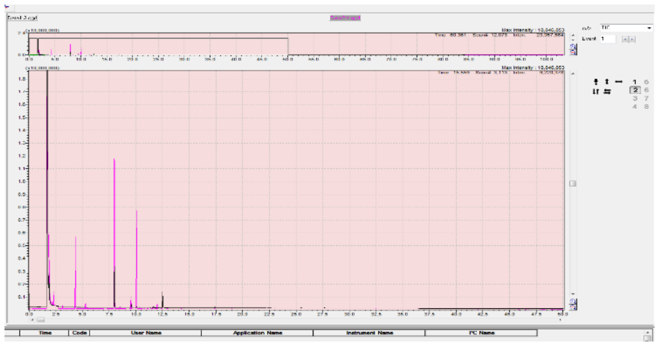

Figure 2: Gas chromatogram of volatile compounds in the peel of *M. indica* (black line) and *M. odorata* (pink line).

Table 4: Volatile compounds in the peel of *M. indica*.

No.	Compound	Retention time (minute)	Concentration (%)
1	Ethylamine	1.687	56.1
2	Ethanol	1.867	16.3
3	Alpha.-Pinene	7.997	7.4
4	Camphene	8.532	0.05
5	beta.-Pinene	9.578	0.6
6	Myrcene	10.09	0.1
7	Cyclotetrasiloxane, octamethyl-	10.339	0.1
8	Phellandrene <beta->	11.679	0.2
9	1,3,6-Octatriene, 3,7-dimethyl-,Z	12.492	2.2
10	Cyclopentasiloxane, decamethyl-	17.416	0.3
11	Cyclohexasiloxane, dodecamethyl-	25.48	0.2
12	Copaene <alpha->	27.658	0.1

Table 5: Volatile compounds in the peel of *M. odorata* .

No.	Compound	Retention time (minute)	Concentration (%)
1	Ethylamine	1.665	46.5
2	Ethanol	1.831	7.4
3	Ethanol	1.863	12.5
4	Ethyl Acetate	2.287	3.1
5	Butyrate <methyl->	3.155	0.3
6	Butanoic acid, ethyl ester	4.335	5.4
7	2-Butenoic acid, ethyl ester	5.295	0.8
8	Butanoic acid, 2-methyl-, ethyl ester	5.434	0.1
9	Alpha.-Pinene	7.986	13.8
10	Camphene	8.503	0.1
11	Beta.-Pinene	9.544	1.2
12	Myrcene	10.076	8.1
13	Terpinene <alpha->	11.117	0.03

Each variety of mango has a specific time for fruit to reach maturity, and these elements have a major influence on weevil infestation. Certain VOCs might act as cues for pests like the mango pulp weevil, signalling the presence of ripe fruit, which is ideal for egg-laying and larval feeding. The mango pulp weevils can distinguish mango species based on their respective maturation periods and suitability for larval development. In comparison to *M. indica, M. odorata* exhibited a higher number of this weevil, indicating that it had more egg depositions. The richness in fibrous tissue found in *M. odorata* probably influenced the egg deposition by *S. firgidus.* This was a reaction to the two-mango species' differing fibrous flesh contents. As results of this study show that *M. odorata* is more fibrous than *M. indica* mangoes, thus suggesting fibrousness could have influenced the attraction patterns observed in gravid females and may also play a role in their preferences. This statement also is supported by the findings of Nor Shazuani (2014), that the gravid females were attracted to Chok Anan mangoes as it is more fibrous compared to other varieties.

Furthermore, both mango types were found to contain a wide variety of volatile chemicals. The level of infection by mango pulp weevil is correlated with the fluctuation of these chemicals. More chemicals were found in *M. odorata* pulp than in *M. indica* pulp, according to the results of the GC-MS study. Mango pulp weevils will also be drawn to the mangoes due to the volatile substances found in their peel and pulp. One type of naturally occurring flavour and aroma chemical found in plants is the amine group (Belitz et al., 2009). Different mango cultivars will result in different chemical volatile compounds (Laohaprasit et al, 2011). The weevils will be drawn in by the strong, sour, fruity smell that this substance emits. Results showed that the peel and pulp of *M. indica* contained the lowest concentration of monoterpene hydrocarbon compared to *M. odorata.* Peel of *M. odorata* contained the highest concentration of this compound. Malundo et al. (1997) stated the flavour of mango was contributed qualitatively and quantitatively by monoterpene hydrocarbon. Moreover, hydrocarbon terpenes such as (Z)-beta-ocimene and myrcene produce the aroma of the flesh of Indian mango cultivars (Batali and Alphonso) (Gholap & Bandyopadhyay, 1997) but it is detected at low concentrations in Thailand mango (Khieo Sawoei) (Tamura et al., 2001). This suggests that pest attraction such as an infestation of mango pulp weevils, might be influenced by the greater concentration of amine groups and monoterpene hydrocarbon molecules. According to Pandit et al. (2009), distinct mango cultivars will have unique volatile chemical compounds that draw pests to the host. Once a chemical attractant is found, insects search for it and move in the direction of the concentration of odour. In this study, Alpha.-Pinene, Myrcene, Camphene, Beta.-Pinene, Phellandrene <beta->, and Copaene <alpha-> were detected in *M. indica* while Limonene, Alpha.-Pinene, Myrcene, Linalool, Camphene, Gamma.-Terpinene, Beta.-Pinene, and Terpinene <alpha-> were detected in *M. odorata.* The mango pulp weevil found in this study would be greatly impacted by all of the attractant substances found. Results show that volatile compounds, such as linalool, which was one of the six component blends that produced 70% appeal to the mango pulp weevil as reported by De Jesus et al. (2004), have attracted more *S. frigidus* to the mango pulp of *M. odorata*. Limonene is another volatile substance that is exclusive to the pulp and peel of *M. odorata* as the most obvious observation from the study. This chemical provides evidence indicating that *M. odorata* has a higher infestation than *M. indica*. According to Alhmedi et al. (2010), limonene can greatly increase *Harmonia axyridis* females' oviposition activity. Next, a higher concentration of pinene and myrcene was found in the pulp and peel of *M. odorata* compared to *M. indica*. According to Masriany et al. (2020), Pinene attracts *Rhodesiella bhutanensis*, *Drosophila* sp., and *Musca domestica*. The aroma of pinene is flowery, herbaceous, piney, and cedar wood-like. Meanwhile, the scent of myrcene is fresh, green, grassy, balsamic, and green mango. Both male and female *Anastrepha obliqua* (Diptera: Tephritidae) have been captured using a trap bait containing myrcene, β-pinene, and (E)-β ocimene (Lopez-Guillen et al., 2011). In addition, β-pinene is one of the scents that attract insects, akin to that of green grass.

4. CONCLUSION

Variations in the amount of volatile compound identified in *M. indica* and *M. odorata* mango were believed to have a relation towards the mango pulp weevil severity of infestation. Because *M. odorata* contains chemicals that attract mango pulp weevils, it has a higher number of larvae, and adults than *M. indica*. Based on the result of GCMS analysis, *M. odorata* pulp and peel have more volatile compounds detected with higher concentrations of Alpha-Pinene (13.8%) compared to *M. indica* (7.4%). Besides, higher infestation level of *S. frigidus* was demonstrated on *M. odorata*, which had higher amine and monoterpene hydrocarbon concentrations. The study found that only *M. indica* contained the compounds phenolandrene and copaene, which were not found in *M. odorata*. *Mangifera odorata* contained linalool, limonene, and terpinene, while *M. indica* did not. This situation occurs because the odour from *M. odorata* peel strongly exposed to the mango pes and becomes a good source for insect attraction, especially *S. frigidus*. In mango crops, these substances work well as pheromone trapping lures or attractants to manage mango pulp weevil populations.

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